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AGENTS, HYDRAZINE AND PHOSPHORUS

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## THE COMPOSITION OF THE TISSUE PROTEINS OF THE RABBIT AS INFLUENCED BY INANITION AND THE HEPATOTOXIC AGENTS, HYDRAZINE AND PHOSPHORUS

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Recent investigations of tissue metabolism in which isotopic nitrogen ( $N^{15}$ ) has been used as a marker have indicated that a "rapid and continuous chemical regeneration of the cell proteins is a general characteristic of living matter" (1). Despite this striking and continuous "chemical activity" of the organ proteins, it is believed that these processes "lead to no final quantitative or qualitative changes" (2) in the composition of the proteins of the tissues. This is in confirmation of the older belief in the constancy of composition of the structural elements of protoplasm, well illustrated by the statement of Osborne and Mendel (3), "That the tissues either form a typical protoplasmic product, or none at all, now seems to be axiomatic in physiology."

This point of view is in sharp contrast to the hypothesis that tissue proteins are variable in composition. It is stated that, in the white rat, quantitative changes in the amino acids of the proteins of the tissues may be effected readily by "protein starvation" (4), by changes in diet, by infections, by toxic agents, by the administration of hormones, and by irradiation (5). In particular, changes in the content of tryptophane, tyrosine, and lysine (4), of cystine and tryptophane (5), and of arginine (6) have been observed. Since the present investigation is concerned only with tissue proteins, the rather extensive literature relating to possible alterations in the composition of the serum proteins will not be reviewed here. This discussion will serve to illustrate the conflict between the observations of Roche (4) and Cahn and Bonot (6), of Schenck and Wollschitt (5), and the older hypothesis of the constancy of the composition of proteins which are essential components of tissues. Lee and Lewis (7) analyzed the mixed proteins of liver, kidney, and muscles of young white rats fasted and fed adequate mixed diets and also diets varying in their content of cystine, one of the amino acids for which lability in tissue proteins has been claimed. No evidence to support the hypothesis that the composition of the proteins of these tissues was influenced by dietary factors, as maintained by Schenck and Wollschitt (5), was obtained. The present paper is concerned with similar studies on young rabbits in a condition of normal nutrition and in

inanition. Since various toxic agents have been stated to alter the structural pattern of tissue proteins (5) and since the liver is an organ importantly concerned in protein metabolism, it was considered of particular interest to study the effect of intoxication produced by two hepatotoxic agents, hydrazine and yellow phosphorus, on the composition of tissue proteins. Under the conditions of the present experiments, no alterations in the quantitative composition of the total tissue proteins of liver, muscle, or kidney, as far as concerns the yield after complete hydrolysis of the amino acids, tyrosine, tryptophane, or cystine, were observed.

#### EXPERIMENTAL

Young albino rabbits in litter units were selected as experimental animals. Each litter of six to seven animals, usually 6 to 8 weeks of age when received in the laboratory, was divided into a control and an experimental group, each consisting so far as possible of equal numbers of animals of the same sex. The animals were fed a commercial mixed food for at least a week prior to the experimental periods. The usual procedure was to kill one animal of each group (control and experimental) in the same week, the age differences between any pair of animals being at most 3 to 4 days. As a rule, two animals, one from each group, were killed each week. The first animals of each litter, when sacrificed, were thus about 9 to 10 weeks of age, and the last from 12 to 13 weeks. In a few instances, the animals were maintained on the commercial mixed food for 6 to 8 weeks before the experiments were begun. The adequacy of this diet was shown by the good growth of the young animals under our laboratory conditions. Although the litters used were separated into groups according to sex, no difference in the results attributable to this factor was observed.

In the first series, a comparison between the proteins of the tissues of well fed and fasted animals was made. The control groups were fasted 5 days, while the experimental groups received the usual diet *ad libitum* for the same period. In the second series, both the control and experimental groups fasted for 5 days, but the animals of the experimental group received 25 mg. of hydrazine (as the hydrochloride) per kilo of body weight subcutaneously on the 4th and 5th days of fasting. The animals were killed at the end of the 5th day.<sup>1</sup> In the third series, yellow phosphorus, suspended and emulsified in warm corn oil, was similarly injected subcutaneously into the animals of the experimental group. Amounts of this solution equivalent to 3.5 mg. of phosphorus per kilo of body weight were

<sup>1</sup> In the blood of the rabbits treated with hydrazine, varying degrees of lipemia were noted. Although histological examinations of the livers were not made, the gross picture was that of moderate fatty infiltration.



injected on the 3rd and 4th days of fasting, and half this amount on the 5th day.<sup>2</sup> The control groups fasted 5 days, as in the other series.

At the conclusion of the final day of the 5 day period, as much blood as possible was removed from the heart with a needle and syringe. The animal was then stunned by a blow on the neck and after exposure of the jugular vein killed by exsanguination. Liver, kidneys, and skeletal muscle of the fore and hind limbs were removed immediately and the protein was prepared from these tissues by the method of Janney (8).

The livers in these experiments always contained small but variable amounts of residual blood. Although the amount of this was believed to be too small to alter significantly the results of the analyses, it was considered desirable to remove as much blood as possible by perfusion of the livers, and to compare the liver proteins thus obtained with those from liver prepared in the usual way. The procedure used for perfusion was essentially that of Luck (9) except that no phosphate was added to the saline used.

The time elapsing between the beginning of the perfusion and the sampling for the preparation of the protein was approximately 1 hour. The muscles and kidneys obtained in these experiments were also analyzed, but perfusion of these organs was not attempted.

The protein obtained from the perfused livers was less highly pigmented than the protein obtained from livers which were not perfused. Analyses showed essentially the same composition of the two preparations of liver protein.

The methods of analysis of the tissue proteins were the same as those previously described (7). Duplicate hydrolyses of the proteins were possible except in a few cases in which the amount of protein available was insufficient (kidneys). With each hydrolysate duplicate or triplicate determinations were carried out. All results are calculated on an ash-free, moisture-free basis.

In Table I are presented the data obtained in the series in which the total coagulable proteins of the tissues of well fed and fasted rabbits were analyzed. In addition, the similar analyses for rat muscle (7) and the composition of myogen and myosin (10) of rabbit muscle, the two best characterized proteins of this tissue, are given. It will be seen that there are no significant differences between the two sets of values, a finding which confirms previous observations on rats from this laboratory (7). The low total nitrogen content of the "protein" prepared from the liver of the well

<sup>2</sup> Pathological examination of the livers of some of these animals showed degenerative changes characteristic of phosphorus poisoning. We wish to express our appreciation to Professor Carl V. Weller, of the Department of Pathology, for his helpful cooperation in examining the tissues.

fed animals by the application of the method of Janney is probably due to the association of the liver proteins with some other protoplasmic component or components in well fed animals. Similar low nitrogen values were reported by one of us (7) in the analyses of liver protein from well fed rats. The existence of a complex of protein and liver glycogen has been postulated (11, 12). The nitrogen contents of the proteins of other tissues and of the livers of fasted animals are satisfactory. The ratio of the nitrogen content to that of the sulfur of the liver protein of the well fed rabbits is within the anticipated range of values and indicates that, whatever the associated substance may be, it probably contains no sulfur or nitrogen.

TABLE I

*Composition of Tissue Proteins of Well Fed and Fasted Rabbits*

The figures represent the averages of analyses of proteins prepared from the tissues of eight animals of each group and are calculated on an ash-free, moisture-free basis.

| Tissue             | Nutrition | Total<br>N      | Total<br>S      | N:S  | Cystine<br>N of<br>total N | Cystine<br>S of<br>total S | Tyrosine<br>N of<br>total N | Tryptophane<br>N of<br>total N |
|--------------------|-----------|-----------------|-----------------|------|----------------------------|----------------------------|-----------------------------|--------------------------------|
|                    |           | <i>per cent</i> | <i>per cent</i> |      | <i>per cent</i>            | <i>per cent</i>            | <i>per cent</i>             | <i>per cent</i>                |
| Liver.....         | Fed       | 12.31           | 0.82            | 15.0 | 1.56                       | 53.8                       | 2.22                        | 1.15                           |
| “.....             | Fasted    | 15.85           | 1.03            | 15.4 | 1.41                       | 50.0                       | 2.09                        | 0.96                           |
| Kidney.....        | Fed       | 16.12           | 1.08            | 14.9 | 1.30                       | 41.5                       | 1.89                        | 0.95                           |
| “.....             | Fasted    | 16.13           | 1.06            | 15.2 | 1.42                       | 48.0                       | 2.03                        | 1.01                           |
| Muscle.....        | Fed       | 15.95           | 0.99            | 16.1 | 1.01                       | 37.3                       | 1.89                        | 0.89                           |
| “.....             | Fasted    | 16.14           | 1.02            | 15.8 | 1.06                       | 38.6                       | 1.93                        | 0.88                           |
| Muscle* (rat)..... | “         | 16.2            | 0.99            | 16.3 | 0.80                       | 30.3                       | 2.13                        | 1.35                           |
| Myogen†.....       |           | 16.6            | 1.29            | 12.9 | 1.37                       | 40.5                       | 1.95                        | 1.25                           |
| Myosin†.....       |           | 16.7            | 1.10            | 15.2 | 0.53                       | 18.6                       | 1.56                        | 0.67                           |

\* Total muscle protein from the data of Lee and Lewis (7).

† Purified protein fractions of rabbit muscle, calculated from the data of Bailey (10).

The analyses of the proteins of the tissues of animals which were fasted and then received hepatotoxic agents, when compared with the analyses of the fasted control groups in each case, also showed no differences in the yield of the amino acid studied (Table II). No evidence is to be obtained from any of the data here presented that the *total coagulable protein* of the tissues is as easily altered in its content of cystine, tyrosine, or tryptophane, as is suggested by Schenck and Wollschitt (5). The data do not rule out possible changes in the distribution of various fractions of liver protein (9), but the composition of the total coagulable protein as prepared by the method of Janney remains unchanged. The data are in confirmation of our previous studies with white rats. It is of interest to note that Wake-



man (13), in 1905, analyzed the livers of dogs receiving hepatotoxic agents and believed that the data suggested a tendency "toward a diminution of the hexone bases as a whole" and, in particular, the arginine content. Subsequently in studies of the liver of dogs similarly treated and in hepatic disease of man (14), he observed changes in the content of hexone bases which were "as a rule comparatively slight" and probably within the limit

TABLE II

*Composition of Tissue Proteins of Fasted Rabbits and of Fasted Rabbits Treated with Hepatotoxic Agents (Hydrazine, Yellow Phosphorus)*

All values are averages for the group and are calculated on an ash-free, moisture-free basis.

| Tissue | Group No. | Nutrition  | No. of animals | Total N         | Total S         | N:S  | Cystine N of total N | Cystine S of total S | Tyrosine N of total N | Tryptophane N of total N |
|--------|-----------|------------|----------------|-----------------|-----------------|------|----------------------|----------------------|-----------------------|--------------------------|
|        |           |            |                | <i>per cent</i> | <i>per cent</i> |      | <i>per cent</i>      | <i>per cent</i>      | <i>per cent</i>       | <i>per cent</i>          |
| Liver  | 1         | Fasted     | 5              | 15.61           | 1.07            | 14.6 | 1.57                 | 51.9                 | 2.12                  | 0.97                     |
|        |           | Hydrazine  | 7              | 15.81           | 1.05            | 15.0 | 1.48                 | 50.9                 | 2.11                  | 1.02                     |
|        | 2         | Fasted     | 4              | 15.55           | 1.09            | 14.3 | 1.43                 | 46.3                 | 2.08                  | 1.04                     |
|        |           | Phosphorus | 6              | 15.86           | 1.14            | 13.9 | 1.34                 | 42.4                 | 2.04                  | 1.08                     |
|        | 3*        | Fasted     | 3              | 15.99           | 1.07            | 14.9 | 1.34                 | 43.7                 | 2.04                  | 1.08                     |
|        |           | Phosphorus | 3              | 15.95           | 1.15            | 13.8 | 1.41                 | 44.2                 | 1.99                  | 1.15                     |
| Muscle | 1         | Fasted     | 5              | 16.10           | 1.08            | 14.9 | 1.12                 | 37.9                 | 2.00                  | 0.94                     |
|        |           | Hydrazine  | 7              | 16.07           | 1.06            | 15.2 | 1.12                 | 38.1                 | 2.06                  | 0.87                     |
|        | 2         | Fasted     | 4              | 16.10           | 1.06            | 15.2 | 1.06                 | 41.3                 | 2.00                  | 1.00                     |
|        |           | Phosphorus | 6              | 15.84           | 1.05            | 15.1 | 1.14                 | 38.0                 | 2.03                  | 1.01                     |
|        | 3*        | Fasted     | 3              | 15.96           | 1.06            | 15.0 | 1.01                 | 35.2                 | 2.03                  | 1.14                     |
|        |           | Phosphorus | 3              | 15.82           | 1.12            | 14.1 | 1.27                 | 39.7                 | 1.98                  | 1.08                     |
| Kidney | 1         | Fasted     | 5              | 16.05           | 1.14            | 14.1 | 1.53                 | 48.2                 | 2.17                  | 0.96                     |
|        |           | Hydrazine  | 7              | 15.98           | 1.12            | 14.3 | 1.45                 | 46.8                 | 2.08                  | 1.01                     |
|        | 2         | Fasted     | 4              | 16.06           | 1.09            | 14.7 | 1.40                 | 38.6                 | 2.11                  | 1.08                     |
|        |           | Phosphorus | 6              | 15.80           | 1.11            | 14.2 | 1.49                 | 47.7                 | 2.14                  | 1.10                     |
|        | 3*        | Fasted     | 3              | 15.69           | 1.15            | 13.6 | 1.50                 | 45.1                 | 2.03                  | 1.24                     |
|        |           | Phosphorus | 3              | 15.68           | 1.22            | 12.8 | 1.50                 | 43.7                 | 2.03                  | 1.24                     |

\* The livers of these animals were perfused as described in the text.

of individual variation. These data were obtained on hydrolysates of liver and not on the hydrolysates of the isolated proteins.

It is realized that the methods for the analysis of various amino acids in protein hydrolysates are imperfect, but it is believed that they are sufficiently accurate to permit comparisons when materials of similar origin and preparation are analyzed.

#### SUMMARY

1. Proteins prepared from the liver, muscle, and kidney of young rabbits by the method of Janney (8) (*i.e.*, total heat-coagulable protein) have been

analyzed for total nitrogen and sulfur, and for the amino acids, cystine, tyrosine, and tryptophane.

2. The composition of these tissue proteins was found to be essentially the same (*a*) in well fed and fasted (5 days) animals and (*b*) in fasted animals in comparison with animals receiving the hepatotoxic agents, hydrazine and phosphorus.

3. The data fail to offer support to the hypothesis of Schenck and Wollschitt (5) that the composition of the tissue proteins is readily altered by changes in the metabolic state of the animal.

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